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Abstract

A time series dataset of macrobenthic invertebrates of Valli di Comacchio lagoon (northern Adriatic) from 1996 to 2015, was analyzed using Biological Traits Analysis, structural indices, AMBI and M-AMBI indices, with a twofold aim to: firstly, test the resilience of the system, and, secondly, test the influence of climate changes, in terms of temperature and precipitation pattern, on macrobenthic dynamics. Along the studied period macrobenthic community showed marked fluctuations, in terms of richness, diversity, biological traits and ecological groups, which could be related with environmental instability of the lagoon. At the same time, a general tendency towards a deterioration of ecological condition of the lagoon was observed, with a general decrease in species richness, diversity, percentage of sensitive species, and a general increase in the proportion of the more opportunistic trait modalities, such as deposit feeders, burrowing, infaunal and short living animals. Increasing yearly temperature explained only a small part of the variability of macrobenthic community, in terms of biological traits and diversity indices, and this was likely due to the effect of natural fluctuations of environmental parameters and anthropogenic disturbance. Nevertheless, all metrics used are consistent in identifying the response of benthic community to a severe disturbance, likely related with the summer heatwave in 2003. Less marked signs of disturbance were observed also in relations to the thermal anomaly of 2012. Biological Traits Analysis combined with more classical structural and ecological indices, proved to be efficient in identifying temporal changes of the community. Our results suggest that the expected increase in frequency, magnitude and duration of heatwaves could pose serious threat to the resilience capacity of lagoonal macrobenthic community.

Keywords	Lagoons; Benthic community; Biological Traits; Heatwave; Adriatic Sea
Taxonomy	Climate Change, Benthic Ecology, Transitional Water

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SIR,

we would like to submit this original manuscript entitled:

Long-term temporal variability of macrobenthic community in a shallow coastal lagoon (Valli di Comacchio, northern Adriatic): is community resistant to climate changes?

by V Pitacco et al to Marine Environmental Research for consideration.

In this paper we use biological traits analysis to assess the effects of climatic changes on lagoonal benthic communties.

Of course, the manuscript has not been previously published, is not currently submitted for review to any other journal, and will not be submitted elsewhere before a decision is made by Marine Environmental Research.

Sincerely yours

M Mistri

Ferrara, December 13, 2017

A 20-yrs time series of benthic invertebrates was analyzed using Biological Traits Analysis

The resilience of the benthic system and the influence of climate changes on it was assessed

An increase in the proportion of the more opportunistic trait modalities was observed

Increasing yearly temperature explained only a small part of the variability of the benthic system

The expected increase in frequency of heatwaves poses threat to the resilience capacity of the studied benthic system

Long-term temporal variability of macrobenthic community in a shallow coastal lagoon (Valli di Comacchio, northern Adriatic): is community resistant to climate changes?

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Abstract

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Keyword: Lagoons, Benthic community, Biological Traits, Heatwave, Adriatic Sea

1. Introduction

Lagoons and estuaries are ecosystems characterized by extremely high biodiversity and high rates of primary productivity. They are transitional areas between land and sea, characterized by a high rate of dynamic changes in the natural environment, but also subjected to degradation due to human population growth and economic development (Lloret et al., 2008). Transitional systems host important socio-economic activities worldwide and their ecosystem services, including fisheries productivity, storm protection, and tourism, are acknowledged as fundamental (e.g.,Mitsch and Gosselink, 2000; Anthony et al., 2009).

Coastal lagoons are also considered to be one of the most fragile marine environments in the face of climate change and its effects (Lloret et al., 2008). According to the different scenarios outlined by the Intergovernmental Panel on Climate Change, an increase in global mean surface air temperature of 1.4 to 5.8 °C relative to values of 1990 is expected by 2100 (IPCC, 2007). Besides, other changes are expected, such as an alteration in rainfall patterns and an increase in the occurrence of extreme climate events, which may lead to a change in the frequency and intensity of storms (Watson et al., 1996) and to heat waves becoming more intense, longer lasting, and/or more frequent (Meehl and Tebaldi, 2004). The Mediterranean area is considered particularly vulnerable, with increasing sea surface warming at two to three times the rate for the global ocean (Vargas-Yáñez et al., 2008) and increasing in the occurrence of hot extremes by 200–500% throughout the region (Diffenbaugh et al., 2007). Those changes could have implications on the circulation and severe impacts on the ecosystems (e.g. fish habitat loss, species extinction and migration, invasive species) (Darmaraki et al., 2017).

Different studies analyzed the vulnerability of transitional ecosystems (coastal lagoons, coastal ponds, river deltas etc.) to climate changes, mainly in terms of sea level rise (Sánchez-Arcilla et al., 1996; Jiménez and Sanchez-arcilla, 1997; Sánchez-Arcilla et al., 1996; Nicholls et al., 1999; Simas et al., 2001; Carbognin and Tosi, 2002). Conversely, few studies provided information on the response of benthic community in transitional ecosystems (Munari, 2011; Lloret et al., 2008).

The Valli di Comacchio is the largest lagoonal ecosystem in the Po River Delta (northeastern Italy) and is located in the coastal area of the north-western Adriatic Sea. This ecosystem was the object of a long-term monitoring campaign for 1996 to 2015. The Valli di Comacchio is a shallow water environment characterized by marked natural variations of both environmental and biological parameters (Munari et al., 2005). According to previous investigations (Munari et al., 2005), the macrobenthic community of the Valli di Comacchio exhibited some degree of both structural and functional redundancy, which led to the hypothesis that the benthic community of the Valli was quite resistant to global environmental changes.

Analysis of temporal trend in benthic community was performed taking into account different aspects of the community: Biological Traits Analysis (BTA) was chosen, together with other, more commonly used, structural and ecological indices, in order to provide a more informative picture of ecological functioning of the lagoon (Bremner et al., 2006). In order to test the response of macrobenthic community in the Valli di Comacchio to climatic changes, in terms of temperature and precipitation pattern, our results were combined with data of temperature and precipitation, to search for a relation among climatic changes and changes of macrobenthic community of the Valli.

Therefore the aim of the present work was twofold: (i) to check for a long-term trend in macrobenthic communities using BTA, structural indices (Richness, Abundance, Shannon and Pielou) and qualitative ecological indices (AMBI and M-AMBI), to test the resilience of the system; (ii) to test whether climatic changes, in terms of temperature and precipitation pattern, could have driven changes in benthic community.

2. Materials and methods

2.1 Study site

The Valli di Comacchio (Fig. 1) are a complex of brackish lagoons whose depth ranges from 0.5 to 1.5m and bottoms are typically muddy. The ecosystem is characterized by limited water renewal (Mistri et al., 2000), being almost completely surrounded by earthen dikes, and separated from the sea by the 2.5 km wide Spina spit. This lagoonal system is connected with the Adriatic Sea by 2 marine channels, the Porto Canale and the Logonovo. Both channels enter the lagoonal complex through the small Valle Fattibello, which also receives a small amount of continental waters through the Navigabile channel, and then flow into the wider basin of Valle Magnavacca (Munari et al., 2003). Occasional marine water inflow may come through the opening of Bellochio drain.

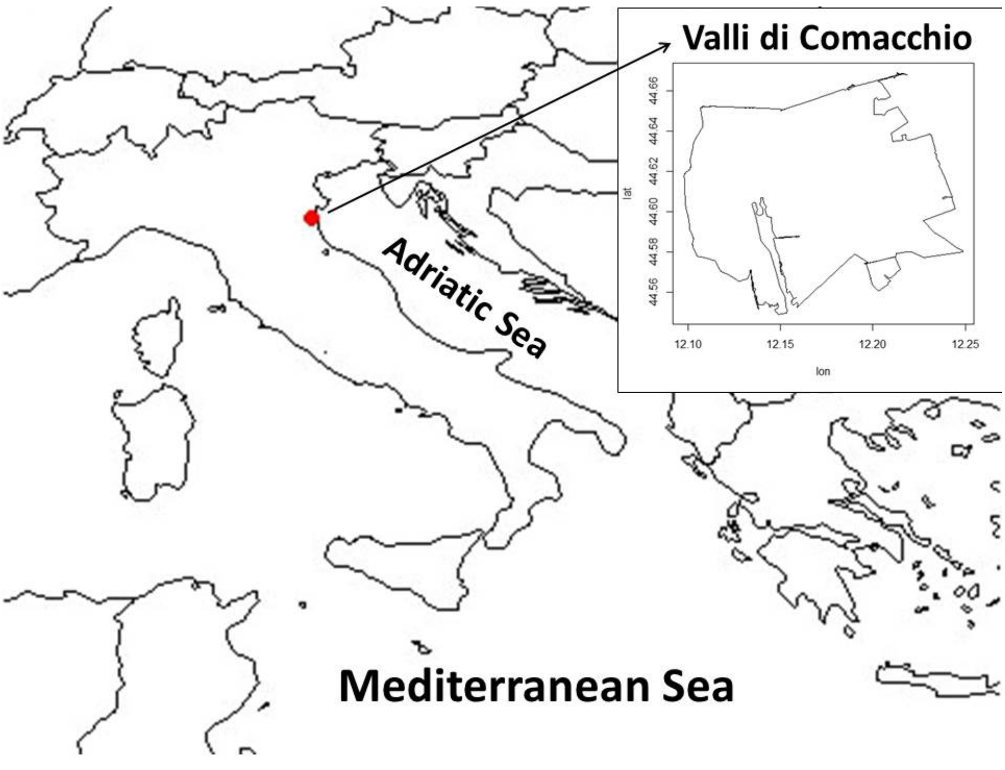

Fig. 1 Map of the studied site

2.2 Collection of samples

Four permanent sampling stations (Fig. 1), being highly representative of ecological conditions found in the Valli (Mistri et al., 2000), were chosen as monitoring stations and studied from 1996 to 2015. At each station three replicates were collected seasonally for macrofaunal community analysis using a Van Veen grab. For the scope of the present work the averages of the three replicates were considered and sampling stations were used as replicates in order to obtain a global picture of the general status of the lagoon. These samples were sieved at 0.5 mm and preserved in 8% formalin. Animals were carefully sorted, identification was performed up to the species level in most cases (exceptions were due to the poor conditions of animals) and all specimens were counted.

2.3 Environmental data

Daily maximum (T_{max}) and minimum temperature (T_{min}), and precipitation (Prec) data for the studied period (1996-2015) were obtained from the archive of the Regional Environmental Protection Agency of Emilia-Romagna (https://www.arpae.it/dettaglio_documento.asp?id=6147&idlivello=1528). Yearly and seasonal average of T_{max}, T_{min} and Prec were calculated. To check for a temporal trend in average yearly environmental parameters, the non-parametric Spearman Rank-order coefficient (r_s) was used (Spearman, 1907). Prior to the analysis a test of autocorrelation was perform in order to test against strong deviations from the assumption of data independence. Since the major effects of global warming on marine organisms are usually observed in the warm season, yearly summer average of T_{max}, T_{min} and Prec were calculated and the deviation from the average of the studied period was calculated for each year. Calculations were performed using R version 2.4.0 (RDevelopmentCoreTeam, 2008).

2.4 Biological traits analysis

After choosing the Biological Traits (BTs) and the modalities to be considered in the analysis (Table 1) the 'taxa by traits' matrix was compiled by gathering data from the literature and selected online databases. Modalities are the different trait categories displayed by organisms. For example, the four modalities of the trait 'feeding' for macrobenthic species are: predator, herbivorous, deposit-feeder, and filter-feeder. The affinity of a taxon for a modality may be ambiguous due to several factors, including (1) interspecific diversity supported by the large morphological and behavioural diversity of organisms or (2) intraspecific diversity due to modifications in life history strategy during the life cycle of an organism (Gerino et al., 2003). Classification was performed through the 'fuzzy coding' procedure (Chevenet et al., 1994). Fuzzy coding allows assessment of affinity of a taxon to multiple categories, using discrete scores from 0 (no affinity) to 3 (total affinity). If the behaviour of a taxon was completely unknown for a trait, it was classified as 0 for all modalities, so that a taxon with such score was not taken into account in the calculation of the column weight (Chevenet et al., 1994).

The number of traits selected for Biological Traits Analysis (BTA) is related to the ability of the analysis to describe the temporal trend of the assemblages studied. Choosing traits to use in a BTA is a compromise between several aspects. For the present study, we considered only traits that could be easily coded, without having to use 0 for all modalities. Nine BTs were chosen related to aspects of life history and habits of the benthic fauna: feeding, mobility, adult life habitat, body size, life span, reproductive technique, type of larva, reproductive frequency and morphology. Each trait was subdivided into a total of 29 variable modalities (Table 1). These traits were used to create the three different numerical matrices required for BTA: (1) matrix ‘taxa by year’ (taxa abundance in each year); (2) matrix ‘taxa by traits’ (biological traits of the taxa); and (3) matrix ‘traits by year’ (the combination of the previous two, biological traits in each year). Data of taxa abundance in the first matrix were transformed by square root in order to reduce the influence of dominant taxa on the samples without losing abundance effects. For multivariate analysis two modalities of the trait “morphology” (exoskeleton and tunic) were treated together, in order to avoid the presence of too many zeros.

Table 1 Biological traits and relative modalities, indices and ecological groups.

(a) Biological traits	Traits modalities	Labels
Feeding	Predator	F/P
	Herbivorous	F/H
	Deposit feeder	F/D
	Filter-feeder	F/F
Mobility	Sessile	M/SE
	Swim	M/SW
	Burrow	M/B
	Crawl	M/C
	Walk	M/W
Adult life habitat	Infauna	H/I
	Epifauna	H/E
Body size	Small (<0.001 g)	B/S
	Medium (0.01–0.05 g)	B/M
	Large (>0.05 g)	B/L
Life span	Short (<1 year)	LS/S
	Medium (1–5 years)	LS/M
	Long (>5 years)	LS/L
Reproductive technique	Asex	RT/A
	Sex: gonocoric	RT/G
	Sex: hermaphrodite	RT/H
Type of larva	None (brooding)	LA/N
	Bentonic	LA/B
	Planktonic – lecitotrophic	LA/L
	Planktonic – planktotrophic	LA/P
Reproductive frequency	Iteroparus	RF/I
	Semelparus	RF/S
Morphology	soft-bodied	MR/SB

	Exoskeleton	MR/EX
	Tunic	MR/T
(b) Biotic indices	AMBI ecological groups	
	Sensitive	EG/I
	Indifferent	EG/II
	Tolerant	EG/III
	2nd order opportunistic	EG/IV
	1st order opportunistic	EG/V

The mean frequencies of modalities within each trait, weighted for taxa abundance, were calculated: (i) for the entire dataset, in order to get the whole picture of the community, (ii) for each season (data not shown), in order to test if the general pattern in traits composition was the same across seasons, (iii) for each year, to search for long-term changes in community structure (only traits showing homogenous pattern across seasons were used).

2.5 Diversity indices and ecological groups

Taxa richness (S), abundance (N), Shannon diversity index on $\log_e$ basis (H) and Pielou index (J), were calculated for each samples, and then yearly averages were calculated. Calculation were performed with PRIMER v6 + PERMANOVA software package, developed in the Plymouth Marine Laboratory (Clarke and Gorley, 2006; Anderson et al., 2008).

Ecological Quality Ratio (EQR) were calculated for AMBI (Borja et al., 2011) and M-AMBI index (Muxika et al., 2007), using the free software (http://www.azti.es v.5.0, species list updated in November 2014) along with the guidelines from the authors (Borja and Muxika, 2005). The percentage of invertebrates belonging to the different ecological groups according to AMBI library (EG, Table 1) at each sampled station was also calculated. Values of EQR was calculated, according to the following reference values: H = 3.3, S = 25 and AMBI = 1.85; and the following thresholds for ES classification: for AMBI ‘‘High’’ if $BC < 1.2$, ‘‘Good’’ if $1.2 < BC \leq 3.3$, ‘‘Moderate’’ if $3.3 < BC \leq 5$, ‘‘Poor’’ if $5 < BC \leq 6$, and ‘‘Bad’’ if $BC \geq 6$ (Borja et al., 2000); for M-AMBI ‘‘High’’ if > 0.96, ‘‘Good’’ if $0.71 <$ M-AMBI ≤ 0.96, ‘‘Moderate’’ if $0.57 <$ M-AMBI ≤ 0.71, ‘‘Poor’’ if $0.46 <$ M-AMBI ≤ 0.57, and ‘‘Bad’’ if M-AMBI ≤ 0.46. Reference values and thresholds for ES classification were those reported by Italian legislation (Act 260/10), for the typology “M-AT-1, non-tidal lagoon”.

2.6 Temporal trend from 1996 to 2015

In order to test if there were significant variations in taxa-abundances, BTs composition, diversity indices and EGs among the different years, permutational multivariate analysis of variance (PERMANOVA; Anderson et al., 2008) was carried out on four matrices created using the whole dataset (square-root transformed ‘taxa by sample’ matrix, square-root transformed ‘traits by sample’ matrix, log-transformed ‘indices by sample’ matrix, and untransformed ‘EGs by sample’ matrix), using a single factor design with 17 levels (corresponding to year of sampling) and the “unrestricted permutation of row data”, as recommended in Anderson et al., 2008.

To provide a general picture of how the community changed with time, according to the different parameters analysed, non-Metric Multidimensional Scaling (MDS) analysis was performed to observe the spacial distribution of the data according to the four different approaches used. Similarity matrices were calculated on: (i) square-root transformed ‘taxa by year’ matrix (taxa abundance in each year); (ii) square-root transformed ‘traits by year’ matrix (biological traits in each year); (iii) log-transformed ‘indices by year’ matrix (diversity indices in each year); and (iv) untransformed ‘EGs by year’ matrix (percentage of EGs for each year). Bray Curtis similarity was used for ‘taxa by year’, ‘traits by year’, and ‘EGs by year’, while Euclidean distance was used for ‘indices by year’ matrix. For each matrix a single MDS was created. In order to compare the different matrices obtained, second stage MDS were used. Second stage MDS (Somerfield and Clarke, 1995), is an ordination technique in which rank correlations between pairs of similarity matrices themselves become the elements of a second similarity matrix, of which gives a summary of the conclusions. Second stage MDS (graphs not shown) were created in order to compare: (i) the four resemblance matrices used for MDS (‘taxa by year’, ‘traits by year’, ‘indices by year’, and ‘EGs by year’); and (ii) Bray Curtis similarity matrices calculated for each BT separately (e.g. ‘feeding by year’, ’mobility by year’, ‘adult life habitat by year’, ‘body size by year’…), and all traits altogether (‘traits by year’ matrix). Spearman correlation coefficients were displayed. Those calculation were performed with PRIMER v6 + PERMANOVA software package (Clarke and Gorley, 2006; Anderson et al., 2008)

In order to identify breakpoints in the multivariate dataset, “Constrained Clustering Analysis” was performed on each of the four matrices: (i) square-root transformed ‘taxa by year’ matrix; (ii) square-root transformed ‘traits by year’ matrix; (iii) log-transformed ‘indices by year’ matrix; and (iv) untransformed ‘EGs by year’ matrix. This technique, originally developed for stratigraphic analysis, is more suitable for time series analysis than ordinary unconstrained cluster analysis, since only adjacent clusters, according to sample order, are considered for merging. As agglomeration method the algorithm “coniss”, using incremental sum of squares (Grimm, 1987) was used. Finally a broken stick model (Bennett, 1996) was used to determine the number of significant groups in the cluster analysis.

For a more detailed visual representation of changes in species abundance and composition, quintiles were calculated on a five-years basis, and a traffic light plot was created for the most frequent species (present with a percentage of frequency higher than 25%), sorted by the 5-year standardised average.

Calculations were performed using the packages ‘vegan’ and ‘rioja’ for R version 2.4.0 (RDevelopmentCoreTeam, 2008).

To check for a temporal linear trend in the mean frequencies of modalities within each BT, in the mean value of each index and in the percentage of each EG the non-parametric Spearman Rank-order coefficient (r_s) was used (Spearman, 1907). Prior to the analysis a test of autocorrelation was perform in order to test against strong deviations from the assumption of independence. Calculations were performed using R version 2.4.0 (RDevelopmentCoreTeam, 2008).

2.7 Relation with environmental variables

Distance-based linear models (DISTLIM) were used to test and quantify the variation in benthic community explained by climatic data. DISTLIM does a partioning of variation in a data cloud described by a resemblance matrix, according to a regression or multiple regression model (Legendre and Anderson, 1999). Yearly average of climatic data (T_{max}, T_{min} and Prec) were used as predictor variables ('climate by year' matrix). The resemblance of the following four matrices were used as response variables: (i) square-root transformed 'taxa by year' matrix; (ii) square-root transformed 'traits by year' matrix; (iii) log-transformed 'indices by year' matrix; and (iv) untransformed 'EGs by year' matrix. Bray Curtis similarity was used for 'taxa by year', 'traits by year', and 'EGs by year' matrices, while Euclidean distance was used for 'indices by year' and 'climate by year' matrices. To test whether found relationships were consistent among different seasons, DISTLIM analysis was performed also for each season separately. All those calculation were performed with PRIMER v6 + PERMANOVA software package (Clarke and Gorley, 2006; Anderson et al., 2008).

For a more detailed understanding of which parameter of the community was influenced most but climatic parameters, the non-parametric Spearman Rank-order coefficient (r_s) was used (Spearman, 1907) to check for correlation among climatic data (T_{max}, T_{min} and Prec) and: (i) the mean frequencies of modalities within each BT; (ii) each structural and ecological index (S, N, H, J, AMBI and M-AMBI) and (iii) each EG. Prior to the analysis a test of autocorrelation was perform in order to test against strong deviations from the assumption of independence. Those calculations were performed using R version 2.4.0 (RDevelopmentCoreTeam, 2008).

3. Results

3.1 Environmental parameters

Along the studied period a moderate increasing linear trend of yearly average maximum temperature T_{max} (r_s = 0.498, $p < 0.05$) and minimum temperature T_{min} (r_s = 0.691, $p < 0.05$) were observed (Fig. 2A). Considering yearly average daily precipitation (Prec) instead, no temporal linear trend was observed ($p > 0.05$). The maximum value of yearly average T_{max} was recorded in 2007 (19.8°C) and the minimum in 1999 (18°C). Yearly average T_{min} instead varied from 8.6°C in 1996 to 11.6°C in 2014. Maximum value of yearly average Prec was recorded in 2010 (2.7 mm), while the minimum value was recorded in 2011 (1 mm). Considering summer data only (Fig. 2B-D), T_{max} showed the highest positive anomalies with respect to the average of the studied period in 2003 and 2012, while the highest negative anomalies were observed in 1997, 1999, and 2004 (Fig. 2B). T_{min} instead showed negative anomalies with respect to the average till 2001, with the highest deviation in 2000. From 2003 yearly values of T_{min} were always above the average, with the exception of 2005, 2007 and 2008 (Fig. 2B). The highest positive deviations were observed in 2003, 2012 and 2015. Precipitations negative anomalies were highest in 2003 and 2012, while the highest positive anomalies were in 2008 and 2010 (Fig. 2D).

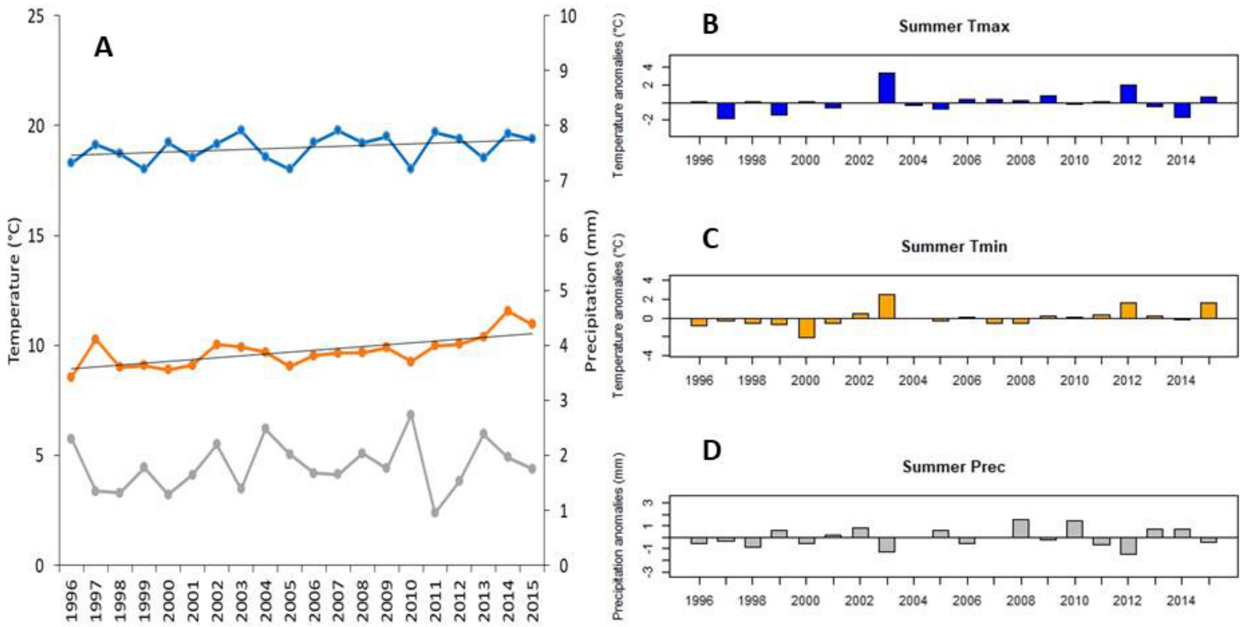

Fig. 2. Variation of yearly average of maximum temperature (T_{max}), minimum temperature(T_{min}) and precipitation (Prec) along the studied period (A). Deviation from the average of the studied period for yearly summer values of T_{max} (B), T_{min} (C) and Prec (D). Data were extracted from ARPAE archive. Legend: blue = T_{max}; orange = T_{min}; grey = Prec.

3.2 General characteristic of Valli di Comacchio lagoons

Altogether along the studied period in Comacchio lagoon 124 different taxa were found. Consequently there were 124 rows in the matrix 'taxa by traits' and the corresponding fuzzy coding procedure involved scoring a matrix of a total of 3596 cells. Taxa belonged to 9 different phyla: Cnidaria, Platyhelminthes, Nemertea, Mollusca, Annelida, Arthropoda, Sipuncula, Echinodermata, and Chordata. Annelida (67 taxa), Arthropoda (31) and Mollusca (10 taxa) were the dominant groups. The most frequent and abundant species along the whole studied period were: the amphipod *Corophium insidiosum*, the polychaete *Streblospio shrubsolii* and oligochaetes of the family Naididae.

In general Comacchio lagoon was characterized by a dominance of deposit feeders (F/D), burrowers (M/B), infaunal (H/I), small sized (B/S), short living animals (LS/S), gonocoric (RT/G), iteroparous (RF/I), soft bodied (MR/SB) animals. The same modalities were dominant in each of the four seasons considered separately. The only exception was the type of larva (LA), that showed a dominance of specie without larval stage (LA/N) in winter and with planktonic planktotrophic larvae (LA/P) in the other seasons. This trait was therefore excluded from following calculations. Considering EGs the lagoon was dominated by tolerant species (EG/III) along the whole studied period.

3.3 Temporal trend from 1996 to 2015

Macrobenthic community showed significant differences in taxa abundances, BTs composition, diversity indices and EGs among different years (PERMANOVA results; Table 2).

Table 2. Results of PERMANOVA analysis.

one factor				
	df	Pseudo-F	P(perm)	P(MC)
taxa	16	4.542	0.0001	0.0001
diversity indices	16	2.704	0.0001	0.0002
BTs	16	2.314	0.0001	0.0001
EGs	16	2.704	0.0001	0.0002

Similarities among different years according to the four aspects of macrobenthic community were displayed in Fig. 3. In Fig. 3A, obtained from 'taxa by year' matrix, a clear segregation of years reflecting a temporal trend could be observed. Changes were not linear and there were years in which the community seemed to go back to the situation of years before, e.g. in year 2006 and 2009 the community seemed to go back to the situation of 2002 and 2003, and in 2014 community seemed to go back to the situation of 2011. Six groups were identified with constrained cluster analysis (appendix 1): 1996-1997, 1998-1999, 2000-2003, 2004, 2005 and 2006-2015. Nevertheless, a clear general tendency towards changes in species abundance and composition from 1996 to 2015 can be observed. From 1999 to 2000 this change was related mainly to change in dominant specie abundances, while from 2002 to 2003 it was mainly due to the disappearance of some species (Fig. 4). The trend that resulted from BTA ('traits by year' matrix, Fig. 3B) showed similar fluctuations: from year 2005 to 2009 the functional structure seemed to go back to the situation of years 2000-2003 and in 2014 the functional structure seemed to regress towards the situation of 2011. Five groups were identified with constrained cluster analysis (appendix 2): 1996-1997, 1998-1999, 2000-2003, 2004-2005 and 2006-2015. Nevertheless there was a general tendency of the functional structure to go back to the situation of 1996, with differences among years less marked. Considering the other two metrics (Fig. 3C-D) instead, the trend was less clear, with fluctuations following a more circular pattern. Diversity indices ('indices by year matrix', Fig. 3C), did not showed clear changes among years, but resulted more constant along the whole studied period. Nevertheless, five groups were identified with constrained cluster analysis (appendix 3): 1996-1997, 1998-1999, 2000-2009, 2011, and 2012-2015. EGs (Fig. 3D) instead showed a clearer temporal trend, with segregation of data reflecting marked fluctuations. From year 2004 to 2009 the composition of EGs seemed to go back to the situation of years 1999-2000, in 2014 EG composition was similar to 2011 and in 2015 it was similar to 2006. Four groups were identified with constrained cluster analysis (appendix 4), and were used as a reference for following analysis: 1996-1999 (period I), 2000-2002 (period II), 2003-2009 (period III), 2011-2015 (period IV). Nevertheless, also in this case a general tendency towards conditions of the '90 could be observed.

Correlation values obtained from the second stage MDS (Table 3) confirmed that the general picture of the temporal trend of the community varied according to the approach used. The approach based on EGs in particular showed no correlation with the other three approaches, whereas the highest correlation was observed between diversity indices and BTs (Table 3).

The different BT analyzed, instead gave more similar results. Correlation values from the second stage MDS based on total BT data ('traits by year' matrix) and data for each single trait considered separately (Table 4), showed high correlations among the pattern obtained with different traits. In particular, the pattern obtained with total BT data showed the highest correlation with the general picture obtained with reproductive technique (RT) (p_s = 0.955), and the lowest correlation with pattern obtained with mobility (M) (p_s = 0.817). The highest correlation value (p_s = 0.965), was found between mobility (M) and adult life habitat (H), the lowest (p_s = 0.678), between mobility (M) and morphology(MR).

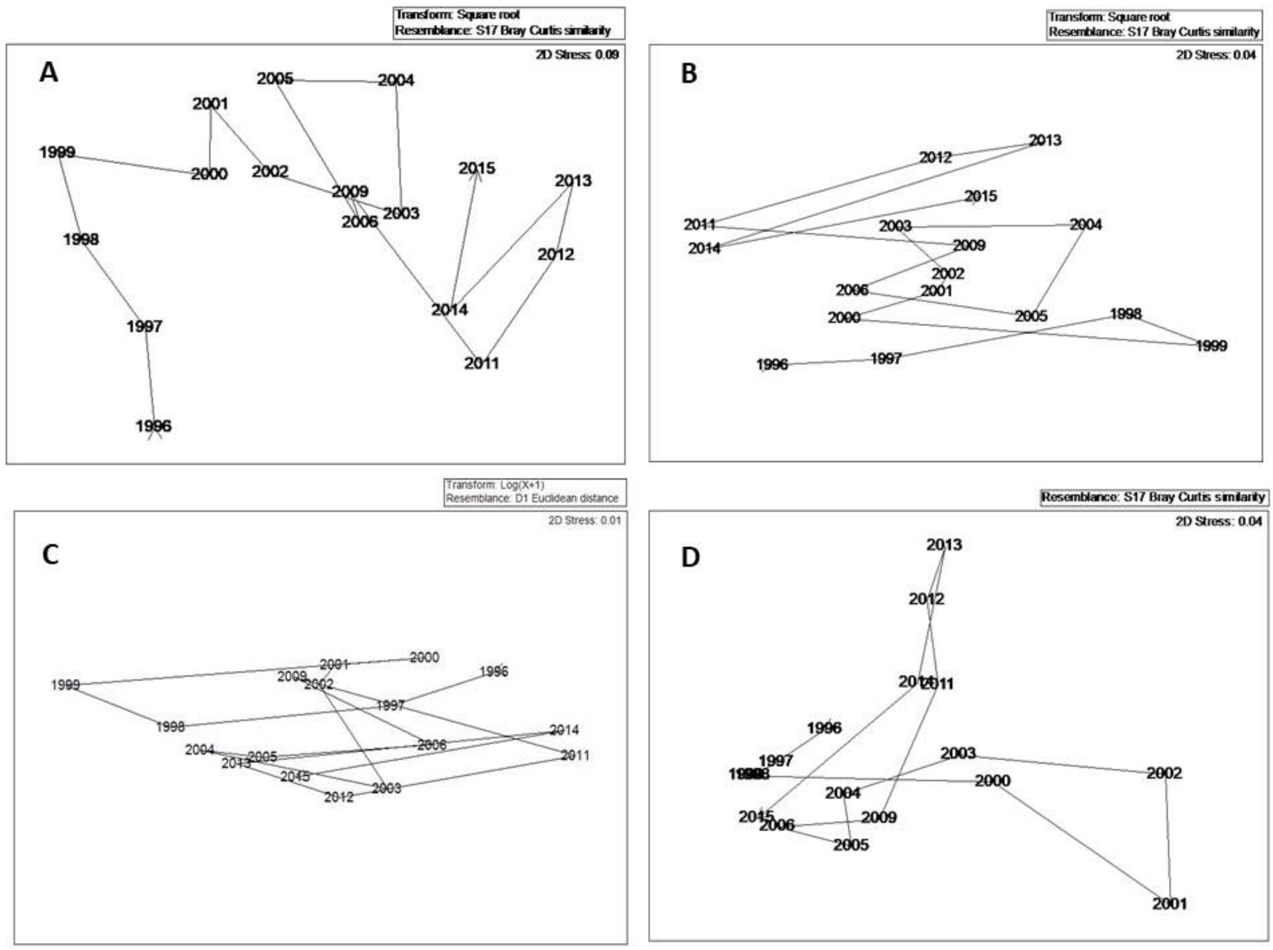

Fig. 3 MDS for species abundances (A), total Biological Traits (B), biodiversity indices (C) and AMBI ecological groups (D).

Table 3 Spearman coefficients of correlation among the different matrices calculated with the second stage MDS.

	taxa by year	traits by year	indices by year
taxa by year			
traits by year	0.532		
indices by year	0.324	0.912	
EGs by year	0.191	-0.075	-0.099

Table 4. Spearman coefficients of correlation among the different matrices with BTs by year calculated with the second stage MDS. See Table 1 for abbreviations.

	traits by year	F by year	M by year	H by year	B by year	LS by year	RT by year	RF by year
traits by year								
F by year	0.908							
M by year	0.874	0.852						
H by year	0.933	0.888	0.965					
B by year	0.899	0.770	0.776	0.853				
LS by year	0.920	0.826	0.912	0.944	0.912			
RT by year	0.955	0.863	0.871	0.937	0.896	0.909		
RF by year	0.928	0.808	0.753	0.845	0.900	0.832	0.898	
MR by year	0.908	0.836	0.678	0.761	0.794	0.748	0.819	0.850

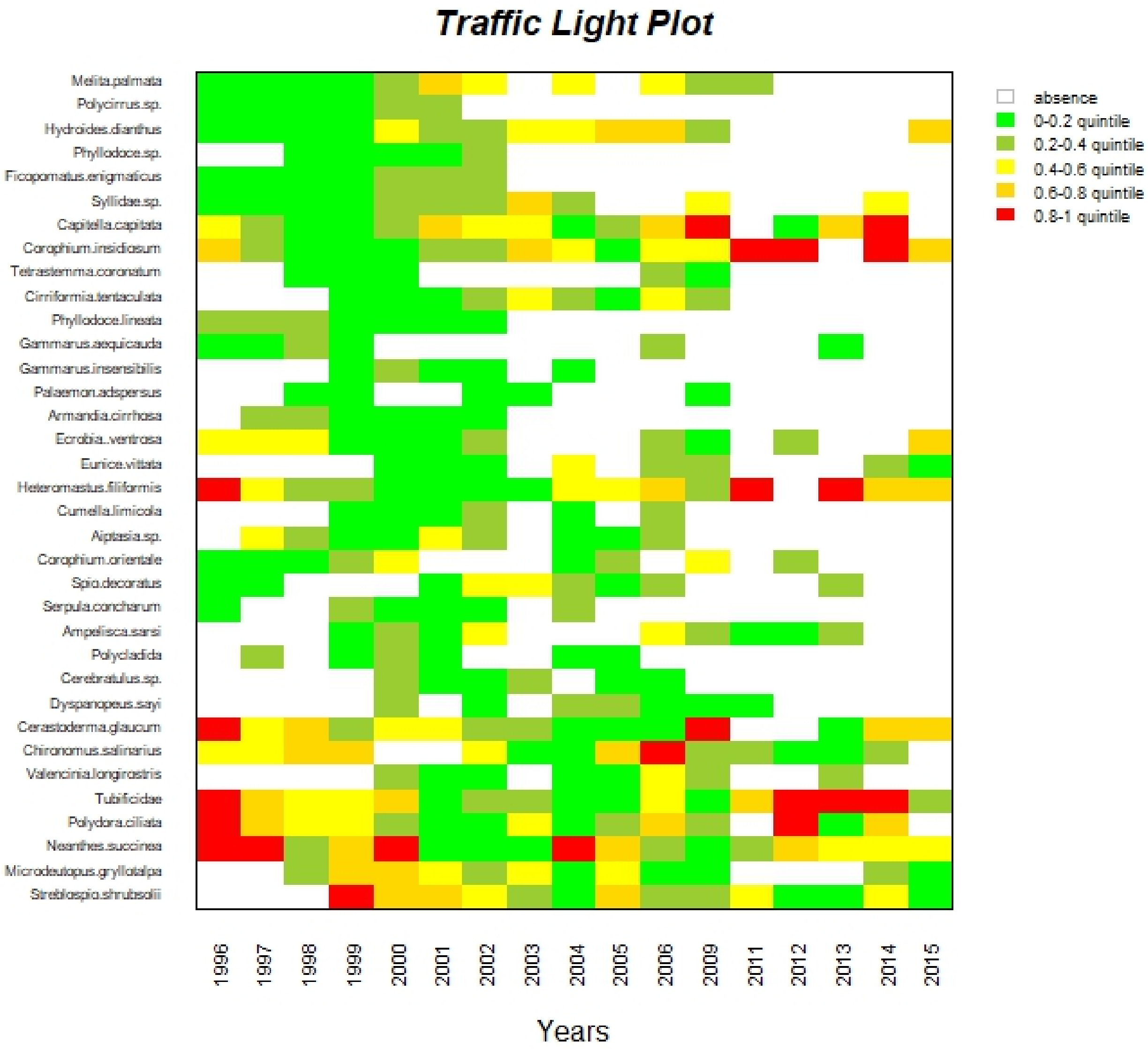

Fig. 4. Traffic light plot of the temporal development of macrobenthic species time-series. Variables are transformed to quintiles, colour coded (red = low values, green = high values), and sorted in numerically descending order according to their abundances.

3.4 BT analysis

The long-term temporal trend showed that the dominant group within each trait remained dominant along the different years of the studied period (Fig. 5), with some exceptions. In particular in year 1996 there was a dominance of sessile (M/SE), epifaunal (H/I) animals, characterized by medium life span (LS/M). In 1999 instead animals with exoskeleton (MR/EX) were dominant instead of soft-bodied animals.

Some traits showed a global linear temporal trend (Table 5Fig. 5). In particular a general change in the proportion of feeding groups (F) was observed, with an increase of the proportion of deposit feeders (D) and the decrease of the proportion of filter feeders (F). Predators (P) and herbivorous (H) also decreased, even if the trend was less marked. Also considering mobility (M), a trend was observed, with an increase of the proportion of burrowing animals (B), and the decrease of sessile (SE) and walking animals (W). The percentage of epifaunal species (H/E) also decreased with an increase of infaunal species (H/I). Fluctuations were observed also considering life span (LS), with two or three maximum for each modality in different years. Overall, there was a general increase of the percentage of short living (S) and a decrease of medium living species (M). Considering reproduction (RT) a general decrease of the percentage of hermaphrodite (H) and an increase of asexual species (A) was observed. Percentage of semelparus species (RF/S) increased and percentage of iteroparus species (RF/I) decreased. Considering body morphology (MR) there was a general decrease in the percentage of species with exoskeleton or tunic (EX or T) and a general increase of the percentage of soft-body species (SB).

Nevertheless from Fig. 5 is evident that such a trend was more of less linear only for reproductive frequency (RF) and reproductive technique (RT). For other traits the temporal trend was clearly not linear from 1996 to 2015, but there were marked fluctuations. Percentages of filter feeders (F), herbivorous (H), sessile (SE), epifaunal (E), medium living (M), and species with exoskeleton (EX) were high from 1996 to 1999 (period I), decreased from 2000 to 2003 (period II), showed a recovery trend increasing from 2004 to 2009 (period IV), and then decreased again from 2011 to 2015 (period V) with the only exception of percentage of species with exoskeleton (EX) that was stable. A slight inversion of this last tendency was observed in 2014 and 2015. The trend of the percentage of deposit feeders (F/D), short living (LS/S), and soft bodied (MR/SB) species instead was opposite. It showed lower values from 1996 to 1999, higher from 2000 to 2003, lower from 2004 to 2009, and higher again from 2011 to 2015. Burrowing species (M/B) instead showed a general increasing trend, with the lowest values in 1996, 1999, 2001, and 2009.

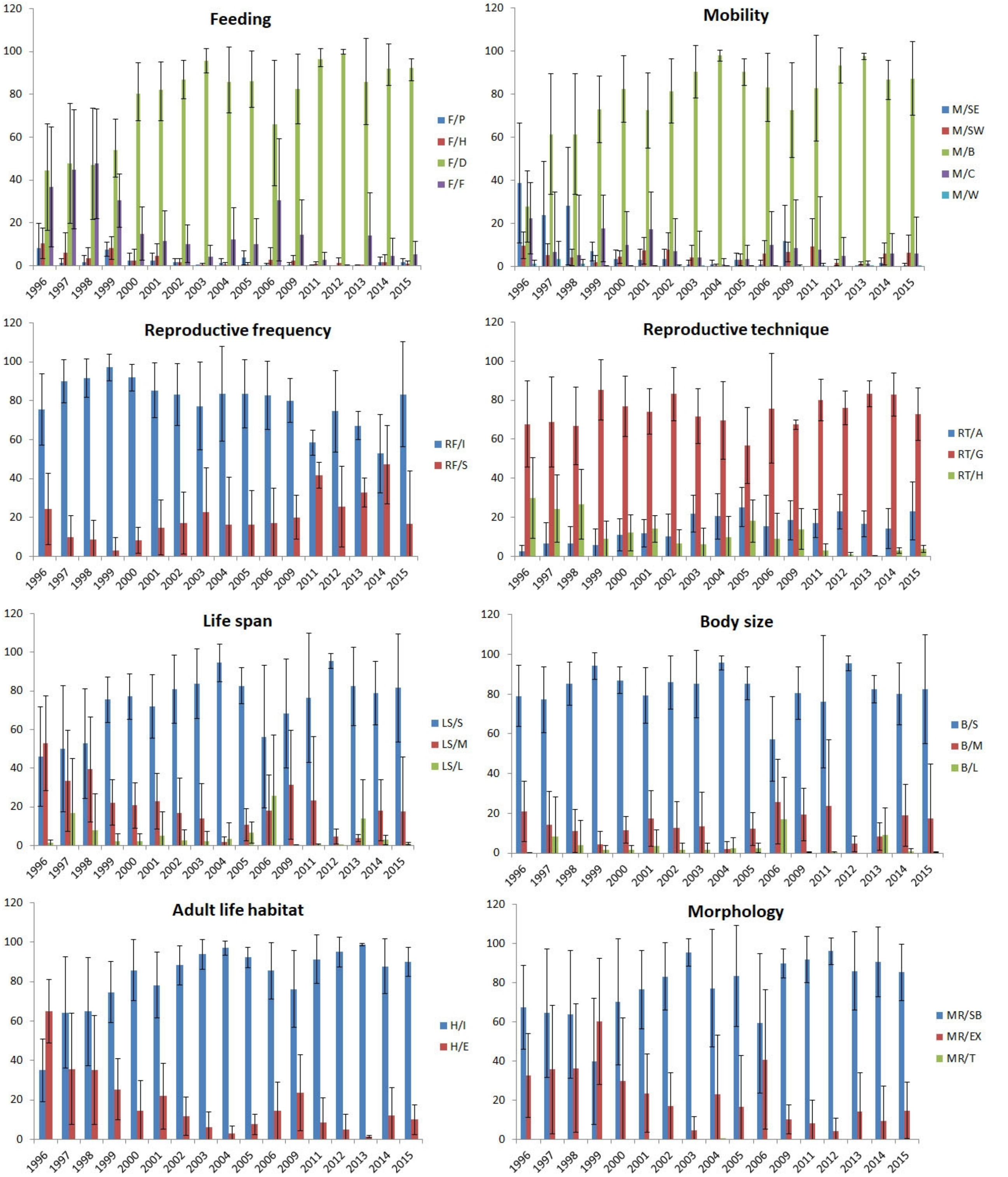

Fig. 5 The mean frequencies of modalities within each trait (±SD), weighted for taxa abundance, calculated for each year. See Table 1 for abbreviations.

Table 5. Spearman correlation coefficients for trends of BTs, indices (S,N, H, J, AMBI and M-AMBI) and ecological groups (EGs) over time and their significancy (see Tab. 1 for abbreviations). Only significant relations are dispayed.

	r_s	*p*		r_s	p
BT/modality			**BT/modality**		
F/P	-0.564	< 0.05	**MR/SB**	0.701	< 0.05
F/H	-0.672	< 0.05	**MR/EX + MR/T**	-0.701	< 0.05
F/D	0.765	< 0.05	**RT/A**	0.74	< 0.05
F/F	-0.686	< 0.05	**RT/H**	-0.775	< 0.05
M/SE	-0.796	< 0.05	**RF/I**	-0.635	< 0.05
M/B	0.701	< 0.05	**RF/S**	0.635	< 0.05
M/W	-0.647	< 0.05	**Index**		
H/I	0.672	< 0.05	**S**	-0.589	< 0.05
H/E	-0.672	< 0.05	**H**	-0.645	< 0.05
B/S	0.869	< 0.05	**M-AMBI**	-0.632	< 0.05
LS/S	0.569	< 0.05	**EG groups**		
LS/M	-0.564	< 0.05	**EG/I**	-0.584	< 0.05

3.5 Diversity indices and EGs

Average values of richness (S) and diversity (H) showed a decreasing trend along the studied period (Table 5), but also in this case the trend was not linear and a certain variability was observed also within years (Fig. 6). The highest values of S were found in 1999 (S = 19 ± 2.7 SD), while minimum values were found in 2003 (S = 7 ± 2.7 SD), 2011 (S = 6 ± 4.2 SD) and 2012 (S = 7.5 ± 1.9 SD). Highest abundance (N) values were found in 1999 (N = 46278± 67164 SD) and lowest in 2011 (N = 1924± 34.9 SD). The highest H values was found in 2001 (H = 1.72± 0.39 SD) and the lowest in 2012 (H = 0.77 ± 0.37 SD). The equidistribution of species (J) was generally quite low, with the highest value found in 1996 (J = 0.80 ± 0.2 SD) and the lowest in 2012 (J = 0.40 ± 0.22 SD).

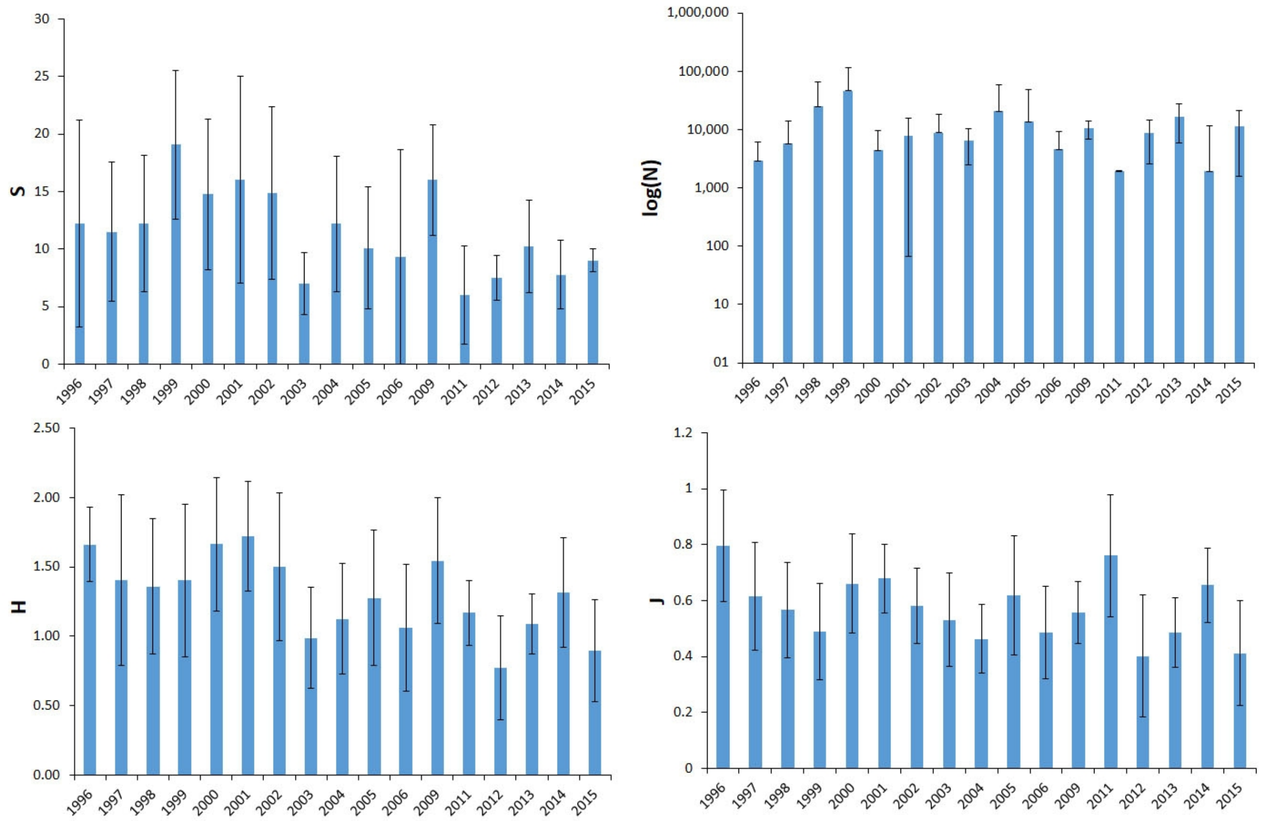

Fig. 6 Diversity indices (S, N, H’, J’) along the studied period. Barplots representing average and standard deviations (SD).

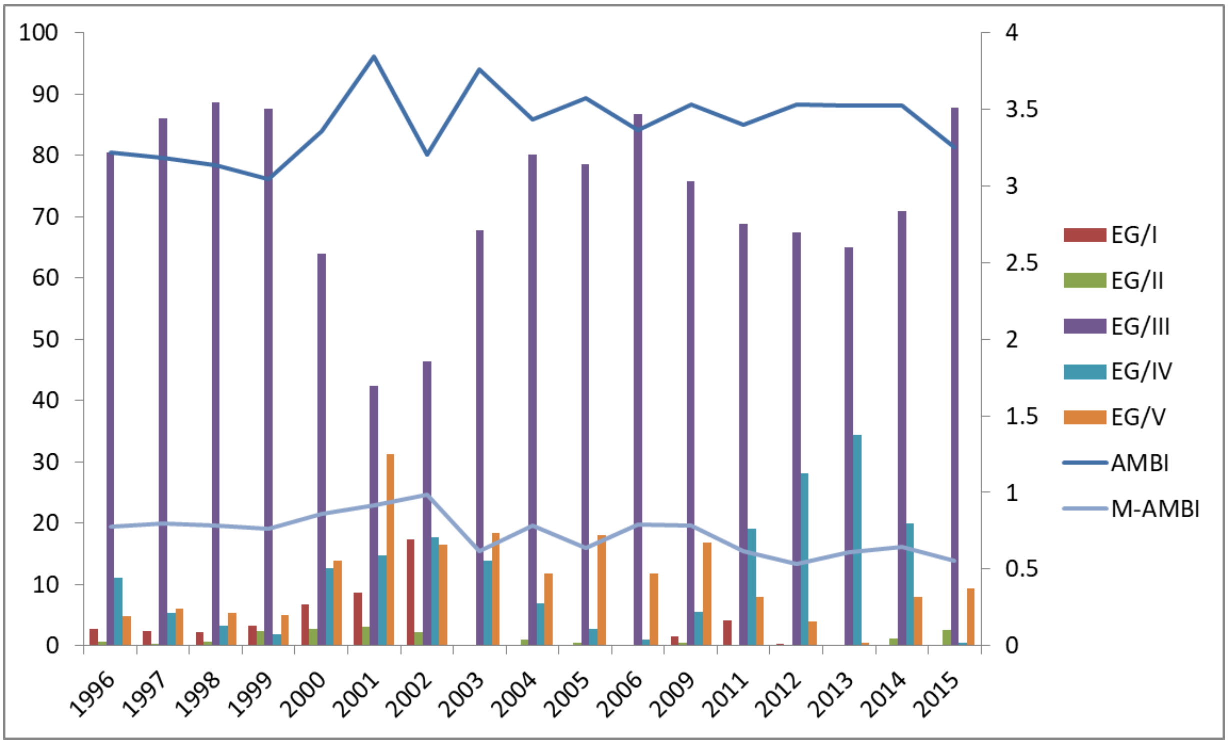

Fig. 7 Percentage of EGs according to AMBI library along the sampling period. EG/I = sensitive species, EG/II = indifferent species, EG/III = tolerant species, EG/IV = second order opportunistic species, EG/V = first order opportunistic species.

Valli di Comacchio lagoon was dominated by tolerant species (EG/III) and this dominance was consistent along the whole studied period (Fig. 7), with percentages varying from 42.3 in 2001 to 88.6% in 1999. Indifferent species were the less represented with low percentages throughout the studied period (0-3.1%). Marked variations instead were observed for sensible (EG/I) and first and second order opportunistic species (EG/V and EG/IV). Sensitive species showed a decreasing trend along the studied period (Table 5). Also in this case fluctuations were observed: sensitive species were present with low percentages (0.1-8.7%) every year, with the exception of 2002, when their percentage was higher (17.4%) and they were totally absent in 2003, 2005, 2014 and 2015. The highest percentages of first order opportunistic species was observed from 2000 to 2009 (11.8-31.2%), while in other periods the percentage was lower (0.5-9.4%). The percentage of second order opportunistic species showed a cyclic pattern along the studied period with maximum values between 2011 and 2014 (19%-34.4%) and secondary maximum values from 2000 to 2003 (12.7%-17.6%). The lowest value was observed in 2015 (0.5%). The best ecological status was observed in 2002, according to the highest M-AMBI value (0.99), and 1999, according to an analysis based only on EGs (lowest AMBI value, 3.05) (Fig. 7). Values of M-AMBI showed a decreasing trend over the studied period (Table 5).

3.6 Relation with environmental factors

Results of DISTLM analysis (Table 6) showed that variations of macrobenthic community considering total species abundances, biological traits and diversity indices were only partially explained by yearly average temperature. A correlation was in fact found between 'taxa by year' matrix and both T_{max} and T_{min}, 'traits by year' matrix and T_{max} and 'indices by year' and T_{max}, but the sequential test showed that temperature explained only a small percentage of total variations: 25% for species abundance data, 26% for BTs and 32% for diversity indices (Table 6). Ecological groups did not showed any significant relationship with climatic parameters. DISTLM analysis performed for each season separately showed that such relationships were significant only for summer periods (Table 6).

Table 6. Results of marginal and sequential tests on parameters that showed significant correlation (DISTLM analysis)

	MARGINAL TESTS		SEQUENTIAL TESTS			
	P	**Prop.**	**R^2**	***P***	**Prop.**	**Cumul.**
Taxa ab						
Tmax	0.01	0.16	0.189	0.003	0.189	0.19
Tmin	0.01	0.19	0.254	0.265	0.065	0.25
taxa ab/summer						
Tmin	0.01	0.23	0.228	0.007	0.228	0.23
BTA tot						
Tmax	0.01	0.26	0.257	0.009	0.257	0.26
BTA tot/summer						
Tmax	0.03	0.34	0.339	0.029	0.339	0.34
Tmin	0.03	0.34	0.390	0.587	0.052	0.39
Diversity indices						
Tmax	0.01	0.32	0.324	0.013	0.324	0.32
Diversity indices/summer						
Tmax	0.03	0.56	0.557	0.025	0.557	0.56
Tmin	0.03	0.52	0.568	0.644	0.011	0.57

Only seven modalities of three different BTs (feeding F, reproductive frequency RF and morphology MR) showed a correlation with both T_{max} and T_{min} (Table 7). T_{max} and T_{min} were positively correlated with percentage of deposit feeders (F/D), semelparus (RF/S) and soft bodied animals (MR/SB), whereas there were negative correlations between T_{max} and T_{min} and percentage of filter feeders (F/F), predators (F/P), iteroparus (RF/I) and animals with exoskeleton (MR/EX) (Table 7). T_{min} showed a negative correlation also with sessile (M/SE) and hermaphrodite (RT/H) (Table 7).

Considering diversity indices, T_{max} was correlated only with S and N (Table 7). T_{min} was correlated with S, and H (Table 7). Precipitations were not correlated with any index ($p > 0.05$). EGs were not correlated with any climatic variable ($p > 0.05$).

Table 7. Spearman correlation coefficients between T_{max} and T_{min} and BT modalities and diversity indices and their significancy (see Tab. 1 for abbreviations). Only significant relations are shown.

	r_s	*p*		r_s	*p*
F/P and Tmax	-0.637	0.01	F/D and Tmin	0.625	0.01
F/D and Tmax	0.610	0.01	F/F and Tmin	-0.515	0.04
F/F and Tmax	-0.537	0.03	M/SE and Tmin	-0.530	0.03
RF/I and Tmax	-0.512	0.04	RT/H and Tmin	-0.708	0.00
RF/S and Tmax	0.512	0.04	RF/I and Tmin	-0.522	0.03
MR/SB and Tmax	0.645	0.01	RF/S and Tmin	0.522	0.03
MR/EX and Tmax	-0.645	0.01	MR/SB and Tmin	0.576	0.02
S and Tmax	-0.603	0.01	MR/EX and Tmin	-0.576	0.02
N and Tmax	-0.525	0.03	S and Tmin	-0.508	0.04
F/P and Tmin	-0.608	0.01	H and Tmin	-0.502	0.04

4. Discussion

4.1 Benthic community of the Valli di Comacchio

The Valli di Comacchio is a harsh environment characterized by shallow waters, limited water renewal (estimate water residence time 115d, Munari et al., 2005), and marked seasonal variations of environmental parameters, such as oxygen, temperature, and salinity (Munari et al., 2005). The community was in fact dominated by tolerant species and the functional structure was quite simplified and dominated by deposit feeders, infaunal, burrowers, soft-bodied, small sized and short living, reproducing gonocorically, several times per year. Such a simplified functional structure is typical of transitional environments, and is consistent with functional structures reported from other Adriatic lagoons (Marchini et al., 2008). One factor that is usually associated with a low functional complexity of the community is the lack of seagrasses. Submerged vegetation acts as a substratum for grazing herbivores, and initiates a cascade of benthic functional groups, as energy source for predators, deposit feeders and filter feeders (Marchini et al., 2008). Unvegetated bottoms are typical of lagoons influenced by the organically enriched inflow of the Po river. In fact a similar functional structure was observed also for two other Po river delta lagoons: Scardovari and Goro (Marchini et al., 2008). In Valli di Comacchio, sparse meadows of the seagrass *Ruppia cirrhosa* occur only in the southern parts of the lagoon (Munari et al., 2005). Earlier studied however reported a higher heterogeneity of the habitats and larger areas of marine phanerogams in Valli di Comacchio at the beginning of the 1970s, and a consequent higher number of macrobenthic species (Colombo et al., 1977). The loss of seagrass beds is often related to increased eutrophication (Cardoso et al., 2004). Unfortunately, there is a gap of knowledge on macrobenthic community from the 1970s to 1999, when the monitoring program started, but it was well documented that from 1985 the lagoon experienced an ecological catastrophe caused by the large load of organic matter discharged from experimental plant for intensive eel aquaculture. It caused mass mortality of benthic fauna, together with the collapse of eel and mullet fishery (Sorokin et al., 1996). Organic enrichment from aquaculture plants ceased in

1990, but signs of disturbance in the macrobenthic community were still detectable after 8 years (Mistri et al., 2000) and signs of amelioration were detected after 11 years from cessation of aquaculture activities (Munari et al., 2003).

4.2 Temporal trend from 1996 to 2015

Macrobenthic community of the Valli di Comacchio lagoons showed clear changes from 1996 to 2015. Those changes were observed at different levels: taxonomic, functional (BTs), structural (diversity indices) and qualitative (ecological indices and EGs).

Different approaches gave different results in terms of differentiating benthic community across the studied period. Nevertheless, every approach clearly showed that temporal trend of benthic community did not followed a linear pattern, but showed a series of fluctuations with some “breaking points” i.e. years in which the community markedly differed from previous year and “turning points”, i.e. years in which the community changed towards a situation similar of some years before. The four approaches were consistent in identifying two breaking points (between 1999 and 2000 and between 2010 and 2011), while the other breaking points, were shifted among the different approaches (form 2002 for EGs to 2003 for others, 2009 for diversity indices and EGs and 2005 for BT and specie abundance).

In the first period, from 1996 to 1999 (period I) the different aspect of the community analysed were consistent in suggesting improving ecological condition of the lagoon. Richness and abundance were high (high S and N) and species were evenly distributed (high J), there was a decreasing trend in the percentage of second order opportunistic species (EG/IV) and there were low values of first order opportunistic species (EG/I). Functional structure was diversified, according to BTA, with an increasing trend of non-dominant, less opportunistic modalities of different traits, such as herbivours, sessile, medium living, epifaunal and species with exoskeleton. This trend towards an amelioration of ecological conditions was likely due to the fact that in this period macrobenthic community was still recovering from the heavy disturbance related with organic enrichment from aquaculture plants (Mistri et al., 2000; Munari et al., 2003).

After the first breaking point, from 2000 to 2002 (period II) the different aspect of the community gave contradictory results. Richness and diversity increased, reaching the maximum values of the whole studied period, and the M-AMBI index reached its highest value, suggesting a clear improvement of ecological condition of the lagoon. There was also an increase in diversity of EGs, with the reduction of the relative percentage of dominant tolerant species (EG/III) and an increase of relative percentages of sensitive (EG/I) species but at the same time also the percentage of opportunistic species (EG/IV and EG/V) increased. The response of the BTs instead was different. The decrease in the percentage of herbivours, sessile, epifaunal, medium/long living species, together with the increasing relative percentage of short living, deposit feeders and soft bodied borrowing species, would suggest a tendency towards a deterioration of ecological conditions. Nevertheless, the dominance of such opportunistic traits, is typical of transitional environments. The high richness and diversity suggested that the disturbance driving such a change had a ‘suitable’ frequency and intensity to establishing a dynamic balance between the rate of competitive displacement and the forces that prevent equilibrium (Munari et al., 2005). Such a dynamic balance allows the continued coexistence of species that would be extinct at competitive equilibrium (Huston, 1979). This would result in a change in species composition, with increasing number of opportunistic species, but at the same time increasing diversity and richness.

The improvement of ecological conditions of Valli di Comacchio in 2001 compared with conditions when the monitoring project start in 1996, was already discussed by Munari et al., 2003, and was related with input of marine waters from the opening of Bellocchio drain (Munari et al., 2003). Nevertheless, since the area considered for this study seemed to be less influenced by this marine water input, the improving of benthic community was more likely simply related to the passing of time after ceasing of disturbance. The present work showed that such an improvement lasted till 2002, when the highest value of M-AMBI of the whole time series was observed.

After the second breaking point in 2003, the response of every aspect of the community was consistent in suggesting a sudden deterioration of ecological conditions. The difference with previous years was marked, in particular considering the reduction in richness and diversity and the total disappearance of sensitive EG/I species. In terms of BTs this was reflected by the highest dominance of opportunistic traits, such as short living, deposit feeders, burrowing, infaunal animals. The consistency of this evaluation across the different aspect of the community analysed, suggested that community in 2003 was affected by a severe disturbance.

In the following period, from 2004 to 2009 (period III) community showed low values of richness and diversity, low percentages of sensitive (EG/I) and indifferent (EG/II), and high percentages of first order opportunistic species (EG/V) and consequent high AMBI and low M-AMBI values. Considering BTs and EGs from 2004 there was a tendency towards an improving of ecological conditions, with a decrease of second order opportunistic species (EG/IV), and increasing percentages of filter feeders, sessile epifaunal, medium/long living species, and crustaceans. Nevertheless, clear signs of recovery were observed only in 2009, with higher values of richness and diversity.

In the last period, from 2011 to 2015 (period IV) the response of every aspect of the community suggested again a deterioration of ecological conditions. There was a decrease of the percentages of filter feeders, sessile, epifaunal, medium living species, and increase of semelparus and asexual species. Even if second order opportunist species (EG/IV) decreased, there was an increase of first order opportunistic species (EG/V). In 2012 in particular the lowest diversity values and the lowest equidistribution were observed, together with the marked reduction of sensitive species (EG/I). There was a slight recovery in 2014 and 2015.

The fluctuations discussed above are consistent with the environmental instability characterizing most of the bottoms of the Valli (Munari et al., 2005). The succession of a community towards recovery after a disturbance is not necessary simple and monotonic, but, because of secondary disturbances, may proceed through progressions and regressions (Karakassis et al., 1999). Fluctuations observed in Valli di Comacchio benthic community suggest that the system had the capacity to recover from disturbance, not only in terms of richness and diversity, but also in terms of functional structure. Previous investigations suggested that the recovery capacity of Valli di Comacchio, could be related to a certain structural and functional redundancy of the community (Munari et al., 2005). It is known that not only species richness, but also functional diversity are insurance against changes (Munari et al., 2005), and in impoverished systems such as transitional environment, species identity and community composition could be more important than species richness for the stability of the system (Faulwetter et al., 2015). The marginal vegetated areas in the southern part of the lagoon, could play a role as well, acting as refugee habitats for macrobenthic communities, when the rest of the lagoon was impoverished, due to anoxia or other destructuring factors (Munari et al., 2003).

Nevertheless, the general trend of some aspects of the community suggests a gradual deterioration of macrobenthic community of the Valli di Comacchio lagoons. Macrobenthic community clearly changed in species composition across the studied period, with a decrease in species richness and diversity, and a decrease of the percentage of sensitive species (EG/I). From the analysis of BTs such a trend was observed within seven traits (feeding, mobility, adult life habitat, life span, reproductive frequency, reproductive technique and morphology), showing a tendency towards a general increase in the percentage of the more opportunistic modalities (i.e. deposit feeders, burrowing, infaunal, short living). Low levels of species richness and diversity and the dominance of pioneering and opportunistic r-selected species, are known to characterize highly stressed estuarine and lagoonal benthic communities (Dauer, 1993). Our concern is related with the fact that if this trend is not going to reverse, the system could lose its recovery capacity. It is well known that benthic communities generally respond rapidly to environmental changes, but the re-establishment of community structure may occur over various time scales and slow recovery processes are quite common (Zajac and Whitlatch, 1982). Given the long recovery periods needed by the community, is therefore essential to continue the monitoring project to fully understand the recovery capacity of the community.

4.3 Relation with environmental factors

The relation between climatic factors and benthic community in transitional systems is complex. In the present work variation in total community, biological traits and diversity indices was only partially explained by the increasing average yearly temperature (T_{max} and T_{min}). The percentage of variability explained was low and the response of benthic community in term of abundances, diversity, and biological traits was limited to warm periods. From BTA only nine out of the 29 analysed modalities showed a correlation with temperature. Climate change is often discussed in relation to a single factor, which is temperature, and models are based on direct physiological response of species to increasing temperature (Galli et al., 2017; Jordà et al., 2012; Rodolfo-Metalpa et al., 2010; Short and Neckles, 1999). Nevertheless, other related factors are known to act synergically, for instance ocean acidification (Rodolfo-Metalpa et al., 2011), hypoxia (Vaquer-Sunyer and Duarte, 2011), invasive species (Kersting et al., 2015) or sea level rise (Short and Neckles, 1999). In transitional areas in particular organisms are subjected to several concurrent factors related with temperature that acted as disturbances. Summer temperatures are known to influence negatively lagoonal benthic community, not just with a direct effect on individual survival, but as a result of their effect on abiotic lagoonal dynamics. In transitional environments, high summer temperatures cause an increase of water temperature, an increase of salinity and a decrease of dissolved oxygen, with values likely to fall to hypoxic conditions (Munari, 2011).

Marked positive thermal anomalies were observed in 2003 and 2012, when both T_{max} and T_{min} were markedly higher than the average of the studied period. In both 2003 and 2012 (as already discussed in the previous paragraph), community showed evidences of deterioration, even if the change was more evident in 2003, with a sudden drop of M-AMBI value, species richness, diversity and the almost total disappearance of both sensitive and indifferent groups. The severe disturbance that affected macrobenthic community in 2003 was likely driven by the thermal anomaly. The heatwave of summer 2003 was long and intense and affected almost the whole western Mediterranean, the Adriatic and central Mediterranean (Marbà et al., 2015), causing mass mortality events and major changes in benthic community in different areas of the Mediterranean (Garrabou et al., 2009; Kersting et al., 2013). Changes in benthic community structure and composition after the heatwave were observed also in Comacchio saltworks (Munari, 2011). Comacchio saltworks are located close to Valli di Comacchio lagoon, but the two areas were not connected and water exchanged with the sea was lower with respect to the area analysed in the present study. Nevertheless in terms of functional structure, the response of the community to the heatwave was similar. In both areas, deposit-feeders became even more dominant, at the expense of herbivorous and, especially, filter-feeders. Long and medium life span organisms decreased, as well as large and medium-sized animals (in our case the effect was more evident in 2004). A decrease in crawling and an increase in burrowing mobility type, as well as a decrease in epifaunal and an increase in infaunal life habitat were also observed in both areas. Nevertheless, in Valli di Comacchio lagoon the change of BTs in 2003 was not abrupt, as it was observed for other parameters, but it resulted more as the final stage of changes started in 2000. Therefore our opinion is that in our case the simplification of functional traits is not a direct consequence of the heatwave, but it could have played a role in reducing the resiliency of the community.

The thermal anomaly of 2012 had a response at level of macrobenthic community, as well. The effect was less marked, and the response was different among different approaches. M-AMBI and EGs proved to be efficient in detecting a change, with the decrease in percentage of sensitive species, and an increase of second order opportunistic species. The reason could be the lower intensity of the anomaly, but also the fact that the ecological status of the community in 2011 was worse, compared with the ecological status of 2002.

The reason why temperature explain just partially the variability of macrobenthic community in Valli di Comacchio, is due to the fact that, like every transitional environment, is subject to natural fluctuations in temperature, salinity and oxygen concentrations and also to important anthropogenic impacts, from land reclamation to the effects of contamination (Mistri et al., 2000), deeply affecting the community. Human intervention could have mitigated or exacerbated the effect of climatic disturbance. The hydraulic management consisted in the opening of Bellochio drain (in 2001 and 2012), enabling marine water to enter the lagoon, and periodic opening of channels connecting the lagoon with Reno river, in the southern part, enabling freshwater inputs.

The management of those channels is functional to fishery activities. They are usually opened in spring, so that fishes are attracted by the decreasing salinity and enter the lagoon. Those occasional freshwater inflows from Reno river and from another channel in the northwestern part, could also be source of contaminants, mainly of agricultural origin. From 1996 to date no remediation measures were taken in order to improve the ecological conditions of Comacchio lagoon, and this factor could also have reduced the resilience of the system.

It is not possible to disentangle the effect of climatic changes from the effects of other anthropogenic impacts. Nevertheless, our results suggested that the increasing frequency and intensity of summer thermal anomalies, consequences of climatic changes, poses the major threat to macrobenthic communities in transitional environment, exacerbating the naturally stressed summer condition of those ecosystems. Moreover seagrasses are vulnerable to climate change as well (Jordà et al., 2012; Short and Neckles, 1999), and given their importance for the recovery capacity of macrobenthic community (Munari et al., 2003), their reduction or disappearance would reduce the recovery capacity of the system even more. According to climatic models heat waves have been increasing in frequency and magnitude in the most recent period and are expected to increase in duration, frequency and spatial extent in the near future (Jordà et al., 2012; Russo et al., 2014; Galli et al., 2017). This would pose serious threat to the resilience capacity of lagoonal macrobenthic community.

4.4 The role of BTA in the view of environmental impact assessment

Biological Traits Analysis (BTA) is an approach recently proposed for describing ecological functioning of marine benthic assemblages. BTA does not measure functioning directly, but uses species biological traits as a proxy for certain ecosystem functioning (Bremner et al., 2003, 2006). In the context of BTA not all traits are as easy to interpret, since the relationship between biological traits and ecological functioning is not always clear. The reason could be that not all biological traits are related with the sensitivity of a species to disturbance. The biological traits most commonly used to describe the response of marine benthic community to different stressors were feeding guilds (e.g. Gaston et al., 1998; Mistri et al., 2000) and body size (e.g. Jennings et al., 2001; Kaiser et al., 2000). According to Marchini et al., 2008, after a comparative study of eight Italian lagoons, beside the traits ‘feeding’ and ‘body size’ also ‘adult life habitat’, and ‘life span’, were ecologically relevant (i.e. showed correlation with EGs). In the present work all those cited traits were useful to identify changes in benthic community over time, with the exception of ‘body size’. This trait in our case could be misleading, since for BTA body size is not calculated on real biomass, but is theoretically based on average values coming from literature. Valli di Comacchio was typically characterized by small size, short living organisms. Also among the biggest species, such as the bivalve *Cerastoderma glaucum*, maximum size was never reached. This species, which in other Mediterranean areas can grow till 3 cm (Derbali et al., 2009; Gontikaki et al., 2003; Tarnowska et al., 2009), was never found bigger that 1cm (*personal observations*), likely because of the high environmental instability of the area (Munari et al., 2005).

On the other side, from the present study also mobility, reproductive technique and reproductive frequency showed variations that enabled a differentiation of benthic communities. Sessile and mobile species are known to respond in different ways to disturbance (Sousa, 1984) and mobility trait proved to be useful in differentiating assemblages also in other marine habitats (Bremner et al., 2003).

In general a complex functional structure is considered signal of the health of the community. The model proposed by Pearson and Rosenberg, 1978 describing the effects of organic pollution on benthic populations, associated high organic load with loss of larger, longer living species (such as filter feeding bivalves or carnivorous polychaetes) and the increasing dominance of more tolerant short-lived opportunists such as small deposit feeders. Paganelli et al., 2012 suggested that riverine inputs have a detrimental effect on community functioning and associated functional complexity with increasing distance from disturbance.

The best way to identify ecological stress, is using multiple methods or analyses with different assumptions, since a single method or analysis is likely to produce stress misclassifications (Dauer, 1993). The consistency of classification between different approaches would provide the robustness necessary to judge the reliability of a stress classification (Dauer, 1993). In the present work the different approaches were consistent in identifying marked fluctuations of community with time, and the effect of asevere stress in 2003. In general the trend observed in BTs gave a more complete picture on community fluctuations, and responded also to lower levels of disturbance, compared with other metrics, confirming the higher sensibility of BTA in highlighting small-scale heterogeneity (Bremner et al., 2003). Since opportunistic traits, increasing in impacted systems, are not necessarily excluded from stable or unimpacted communities (Townsend et al., 1997), investigating changes in the relative proportions of biological traits over time may provide the only reliable means to identify impact-driven alterations to ecological functioning (Bremner et al., 2003). In our case, the combination of BTs with EGs, AMBI, M-AMBI and diversity indices was essential for a correct classification of the ecological status of the lagoon and for a clear view of temporal trend of macrobenthic community.

The major difficulty in performing BTA involves the amount of time and information required to compile a dataset of species traits. Furthermore, gaps in the knowledge of species biology, particularly critical for the marine benthos, may still limit the power of these types of analyses (Marchini et al., 2008). Nevertheless, in view of climate changes, given the impossibility of maintaining the environmental status quo in transitional areas, management actions in lagoons should aim at preserving ecosystem services despite climate-driven environmental changes (Chapman, 2012; Watson et al., 1996). In this view, to understand long-term functional aspects of macrobenthic community is critical and the BTA, combined with classical approaches, applied to long-term data series could result a useful tool.

5. Conclusions

The functional structure of the macrobenthic community of Valli di Comacchio lagoon was quite simplified and dominated by tolerant species, with a dominance of deposit feeders, infaunal, burrowers, soft-bodied, small sized and short living animals. Along the studied period, from 1996 to 2015, community showed marked fluctuations in species composition, richness, diversity, functional structure and ecological groups, as typically observed in environments characterized by high environmental instability. In 2003 the community was strongly affected by a severe disturbance, probably driven by the summer heat wave. Less marked signs of disturbance were observed also in relations to the thermal anomaly of 2012. After those disturbance events there were signs of amelioration, suggesting a certain resilience of the community to environmental changes. Nevertheless, a general trend towards a deterioration of the community was observed, with a general decrease in species richness, diversity, percentage of sensitive species, and a general increase in the proportion of the more opportunistic trait modalities, such as deposit feeders, burrowing, infaunal and short living animals. Such a trend suggests that the community was not able to recover completely, and this could be related with the high intensity and/or the frequency of disturbance. In view of the expected increase in frequency and magnitude of climatic extreme events, such as heatwaves, the resilience capacity of the community could be affected even more.

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